Current Biology 21, 1968–1978, December 6, 2011 ©2011 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2011.10.051

Article

SIN-Inhibitory Phosphatase Complex Promotes Cdc11p Dephosphorylation and Propagates SIN Asymmetry in Fission Yeast

N. Sadananda Singh,[1] Nan Shao,[1,2] Janel R. McLean,[4] Mayalagu Sevugan,[1] Liping Ren,[4] Ting Gang Chew,[1] Andrea Bimbo,[1] Reetu Sharma,[1,2] Xie Tang,[1] Kathleen L. Gould,[4] and Mohan K. Balasubramanian[1,2,3,*]

[1]Temasek Life Sciences Laboratory
[2]Department of Biological Sciences
[3]Mechanobiology Institute
The National University of Singapore, 1 Research Link, Singapore 117604, Singapore
[4]Howard Hughes Medical Institute and Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

Summary

Background: Cytokinesis in many eukaryotes involves the function of an actomyosin-based contractile ring. In fission yeast, actomyosin ring maturation and stability require a conserved signaling pathway termed the SIN (septation initiation network). The SIN consists of a GTPase (Spg1p) and three protein kinases, all of which localize to the mitotic spindle pole bodies (SPBs). Two of the SIN kinases, Cdc7p and Sid1p, localize asymmetrically to the newly duplicated SPB in late anaphase. How this asymmetry is achieved is not understood, although it is known that their symmetric localization impairs cytokinesis.

Results: Here we characterize a new Forkhead-domain-associated protein, Csc1p, and identify SIN-inhibitory PP2A complex (SIP), which is crucial for the establishment of SIN asymmetry. Csc1p localizes to both SPBs early in mitosis, is lost from the SPB that accumulates Cdc7p, and instead accumulates at the SPB lacking Cdc7p. Csc1p is required for the dephosphorylation of the SIN scaffolding protein Cdc11p and is thereby required for the recruitment of Byr4p, a component of the GTPase-activating subunit for Spg1p, to the SPB.

Conclusions: Because Cdc7p does not bind to GDP-Spg1p, we propose that the SIP-mediated Cdc11p dephosphorylation and the resulting recruitment of Byr4p are among the earliest steps in the establishment of SIN asymmetry.

Introduction

Cytokinesis is the terminal step in cell division during which a single mother cell is physically divided into two daughters. Cytokinesis in many eukaryotes, ranging from yeasts and fungi to metazoans, involves the function of an actomyosin-based contractile ring. The actomyosin ring is assembled upon entry into mitosis and constricts following completion of anaphase. Although detailed mechanisms regulating actomyosin ring positioning and assembly have emerged in recent years [1–3], the molecular controls that ensure that cytokinesis occurs only once per cell cycle and precisely after chromosome segregation are not understood.

*Correspondence: mohan@tll.org.sg

Over the last two decades, the fission yeast *Schizosaccharomyces pombe* has emerged as an attractive model for the study of cytokinesis because it divides using an actomyosin ring and because there are well-developed experimental approaches applicable to this organism [2, 4, 5]. In fission yeast, a signaling cascade termed the SIN (septation initiation network) plays a key role in the regulation of cytokinesis [6–8]. Fission yeast SIN-like signaling cascades have been identified in budding yeasts (termed the MEN [mitotic exit network]), fungi, and metazoans [3, 6, 7, 9, 10]. SIN includes three protein kinases (Cdc7p, Sid1p, and Sid2p); a small GTPase (Spg1p); a pair of spindle pole body (SPB) resident proteins, Cdc11p and Sid4p; and binding partners of Sid1p and Sid2p, termed Cdc14p and Mob1p, respectively [9, 11–20]. Loss-of-function mutations in any of these genes lead to defects in actomyosin ring maturation, constriction, and septation and to the generation of multinucleate cells [9, 12, 14–16, 18, 20]. By contrast, ectopic activation of the SIN through mutations in the two-component GTPase-activating protein (GAP) for Spg1p (Cdc16p and Byr4p are the subunits) [21–24] or upon overexpression of Spg1p [18] leads to uncontrolled septation without intervening mitosis. These and other observations have led to the idea that the SIN coordinates chromosome segregation with cytokinesis [5, 15, 25–29].

Protein localization studies have shown that all SIN components localize to the SPB in a manner dependent on the proteins Cdc11p and Sid4p [11, 19, 30]. Of these, Spg1p, Sid2p, and Mob1p are detected at the SPB throughout the cell cycle [9, 16, 18, 20], whereas Sid1p, Cdc7p, and Cdc14p localize to only one of the two SPBs (the newly generated SPB) during late mitosis and do not localize to the SPB in interphase [14, 15, 31]. Innovative cell biological experiments have shown that Cdc7p, Sid1p, and Cdc14p reside on the SPB containing GTP-bound Spg1p [31, 32]. Conversely, Byr4p and Cdc16p localize to the SPBs throughout interphase and then specifically to the old SPB during late mitosis [25, 31–33]. Importantly, the ectopic localization of Cdc7p, Sid1p, and Cdc14p [15] [32] to either the interphase SPB or to both SPBs in mitosis correlates with repeated rounds of septation in the absence of intervening mitosis. These findings have led to the proposal that hyperactivation of the SIN (via the symmetric localization of Cdc7p, Sid1p, and Cdc14p) leads to defective cytokinesis characterized by uncontrolled septation [21, 22, 32]. However, the molecular mechanisms behind the generation of asymmetry in the localization of these proteins are not fully understood.

In this study, we identify SIN-inhibitory PP2A complex (SIP), containing at least six proteins, including a previously uncharacterized catalytic subunit of protein phosphatase 2A (Ppa3p) and the A subunit of the PP2A holoenzyme Paa1p. SIP components localize to the SPBs early in mitosis, transiently localize asymmetrically to the old SPB, and promote localization of Byr4p to the old SPB. This in turn leads to the establishment and propagation of Cdc7p and Sid1p at the new SPB.

Results

Csc1p Localizes to the SPB in Early Mitosis

Because all known SIN components localize to the SPBs during mitosis, we hypothesized that additional new regulators

of the SIN could be identified by characterizing proteins that specifically localize to the mitotic SPBs [6, 7, 34, 35]. To this end, we searched the GFP-ORFeome [36] and identified a previously uncharacterized Forkhead-associated domain protein (SPBC3H7.13), which we have named Csc1p (*c*omponent of *S*IP *c*omplex *1*). Csc1p is a 301 aa protein that contains an N-terminal FHA domain (Figure 1A) and is most related to *Saccharomyces cerevisiae* Far10p (data not shown).

To further analyze the localization of Csc1p, we generated a Csc1p-GFP-expressing strain. We deemed the Csc1p-GFP to be fully functional based on criteria described in a subsequent section. Csc1p-GFP was detected at both SPBs in early mitotic cells (Figure 1B) but was not clearly detected on either of the SPBs following initiation of anaphase B (data not shown). We utilized several conditional mutants to further analyze its localization. Csc1p was not detected on the SPBs in G2-arrested *cdc25*-22 cells [37] (Figure 1C) but was detected on the nonseparated SPBs in prometaphase-arrested *nda3*-KM311 [38] cells lacking a mitotic spindle (Figure 1C). Localization of Csc1p to the SPBs did not require signaling through the SIN, because Csc1p localization was unaffected in the SIN scaffold mutants *cdc11*-123 and *sid4*-SA1 and the downstream kinase mutant *sid2*-250 (Figure 1C; data not shown for *cdc11*-123 and *sid2*-250). It has been shown previously that SIN components require the SPB-resident protein Ppc89p for their localization to the SPB [39]. We therefore tested whether Ppc89p was required for the SPB localization of Csc1p. To this end, we expressed Csc1p-GFP in a yeast strain in which the *ppc89* gene was placed under control of the thiamine-repressible *nmt81* promoter. In this strain, upon growth in repressing conditions, Csc1p-GFP failed to localize to the SPBs in approximately 37% of the cells (Figure 1C). In other experiments, we found that the localization of a Csc1p-interacting protein (Csc3p; described in a later section) was fully abolished in germinated spores deleted for *ppc89*, establishing that the localization of Csc1p (and associated factors) to the SPB depends on Ppc89p. Collectively, these experiments established that Csc1p localizes to the SPB early in mitosis in a Ppc89p-dependent manner and exits the SPB upon anaphase progression.

Csc1p Becomes Asymmetrically Localized Transiently to the Mother SPB Not Containing Cdc7p

In some cells, Csc1p displayed a slight asymmetry in localization before disappearing from both SPBs. To address whether Csc1p indeed exhibited a transient asymmetric localization at the SPBs, we studied its localization in synchronous *cdc25*-22 mutant cells, which undergo a prolonged mitosis with a mitotic spindle nearly two and a half times longer than that in wild-type cells. From this analysis, it was apparent that approximately 54% of cells showed unequal distribution of Csc1p to one of the two SPBs (Figure 1D). Because several components of the SIN are known to localize to one of the two SPBs [14, 15, 17, 22], we tested whether Csc1p levels were higher on the new SPB containing the SIN effector kinases Cdc7p and Sid1p [31] or on the old SPB that does not contain these effector kinases. To investigate this, we generated a strain expressing Csc1p-GFP and Cdc7p-mCherry. Csc1p was detected in two patterns: in ~57% of cells, Csc1p signal was of equal intensity on both SPBs, and in ~43% of cells, Csc1p signal was weaker or absent on one SPB and stronger on the other SPB. In cells containing Csc1p signals of equal intensity, Cdc7p also localized to both SPBs with equal intensity (Figure 1E). Interestingly, when Csc1p displayed unequal localization, Cdc7p signal intensity was reciprocal to that of Csc1p (i.e., if Csc1p levels were high on one SPB, Cdc7p levels were low on that SPB and vice versa).

Because many of our studies utilized the *cdc25*-22 mutant, albeit at permissive temperature, it was possible that the asymmetry was an artifact of the *cdc25*-22 mutation. To rule out this possibility, we investigated the localization of Csc1p in otherwise wild-type cells. Consistent with our previous studies, Csc1p was asymmetrically localized in approximately 54% of cells with a short mitotic spindle (Figure 1F; two cells in the bottom; short spindle defined as those less than 5 μm in size), whereas in the rest of cells with short spindles, Csc1p was symmetric in localization (Figure 1F; one cell on top). Based on these results, we conclude that Csc1p localizes to early mitotic SPBs and then is redistributed at the onset of anaphase such that Csc1p and Cdc7p segregate to opposite SPBs.

We performed time-lapse analysis of the distribution of Csc1p-GFP and Cdc7-mCherry to gain better insight into the dynamics of these proteins in relation to each other. Time-lapse imaging of >10 cells expressing Csc1p-GFP and Cdc7p-mCherry further confirmed that Csc1p-GFP and Cdc7p-mCherry segregate to opposite SPBs (Figure 1G; note time points 6′ and 8′, indicated with an arrow, when asymmetry of Csc1p and Cdc7p is established and propagated). Interestingly, we found in these studies that Csc1p was fully lost from the SPBs only when the mitotic spindle was approximately 7–8 μm in length (Figure 1G; time points 8′ and 10′).

Our analysis of fixed cells revealed that Cdc7p and Csc1p asymmetry was apparent in cells with short spindles, although complete loss of Csc1p from the SPB occurred in the mid-anaphase B. Degradation of the mitotic B-type cyclin Cdc13p (and cyclin-dependent kinase [CDK] inactivation) is completed only in the middle of anaphase B [26, 40]. Therefore, we considered the possibility that removal of Csc1p from the SPB might be initiated at onset of cyclin B proteolysis and completed upon full degradation of Cdc13p. We could not test the relationship between CDK inactivation and Csc1p localization to the SPB because Csc1p-GFP fluorescence was dampened at 36°C, the restrictive temperature for *cdc13*-117 [41] mutants. However, the localization of a Csc1p-associated protein (Csc3p, described in a later section) to the SPB was lost when metaphase-arrested *cdc13*-117 cells were shifted to the restrictive temperature to inactivate CDK function (see Figure S1A available online; metaphase arrest was achieved by overproduction of the mitotic checkpoint protein Mad2p in *cdc13*-117 cells cultured at the permissive temperature). Furthermore, Csc1p was detected at the SPB in a high proportion of cells with elongated mitotic spindles in cells expressing a Cdc13Δ81, a nondegradable version of Cdc13p (Figure S1B). These experiments strongly suggested that the removal of Csc1p and associated factors from the SPB is linked with cyclin B degradation.

Csc1p Is a New Negative Regulator of the SIN

To check whether *csc1* functions in cytokinesis, we generated a gene-deletion strain and investigated the phenotype. In wild-type cells, septum deposition was seen only in binucleated cells that had completed mitosis. However, in cells deleted for *csc1*, a low proportion of uninucleate cells displayed division septa (Figure 2A; <1% of n > 500). In addition, binucleate cells with more than one septum were also detected (Figure 2A). The presence of uninucleate cells with a septum and binucleate cells with more than one septum suggested that

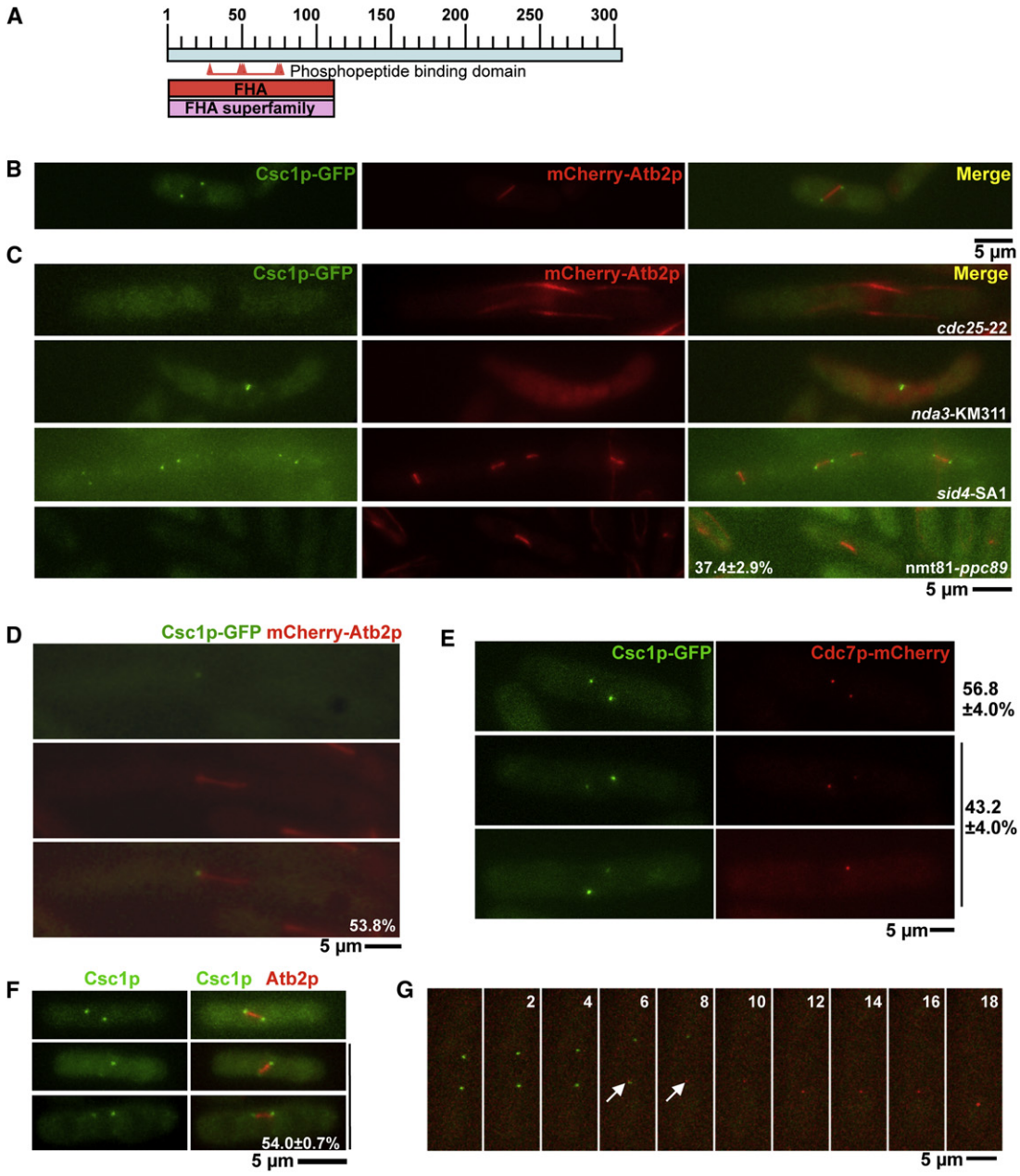

Figure 1. Csc1p, a New Forkhead-Associated Domain-Containing Protein, Localizes to Both Spindle Pole Bodies and Then Transiently to One Spindle Pole Body during Early Mitosis

(A) Schematic representation of Csc1p with the FHA domain at the N terminus. The phosphopeptide binding domain is highlighted between amino acids 30 and 80.

(B) Csc1p localizes to both spindle pole bodies (SPBs) in cells with short mitotic spindles. *csc1-GFP mCherry-atb2* cells were grown at 24°C until mid-log phase and fixed with methanol, and the localization of Csc1p was imaged.

(C) Csc1p localization depends on entry into mitosis and the SPB component Ppc89p but does not require microtubules and SIN activity. *cdc25*-22 cells and sid4-SA1 cells expressing Csc1p-GFP and mCherry-Atb2p were grown at 24°C, shifted to the semipermissive temperature of 33°C for 5 hr, and imaged for the two fluorescent proteins. *nda3*-KM311 cells expressing Csc1p-GFP and mCherry-Atb2p were grown at 30°C, shifted to the nonpermissive temperature of 19°C, and imaged for the two fluorescent proteins. *nmt81-ppc89* cells expressing Csc1p-GFP and mCherry-Atb2p were grown in Edinburgh minimal medium containing thiamine for 36 hr. The cells were then fixed with methanol and imaged for the two fluorescent proteins (n = 50 cells from each of three independent experiments).

(D) Csc1p shows a transient asymmetric localization to one SPB. *cdc25-22* cells expressing Csc1p-GFP and mCherry-Atb2p were arrested at G2 by incubation at 33°C for 3 hr. The cells were then released at 24°C and grown for 1 hr. Cells were fixed with methanol, and the localization of Csc1p and microtubules was imaged. The percentage of cells with asymmetric distribution of Csc1p is shown at the bottom of the figure (n = 26).

(E–G) Csc1p and Cdc7p segregate to opposite SPBs.

(E) *cdc25-22* cells expressing Csc1p-GFP and Cdc7p-mCherry were grown at the permissive temperature and fixed with methanol. The fluorescence patterns of Csc1p and Cdc7p were scored. Cells that showed signals of both Csc1p and Cdc7p were imaged to analyze the localization relationship of the two proteins at SPBs. The percentages of cells showing symmetric (top panel) and asymmetric (bottom two panels) distribution of Cdc7p and Csc1p are indicated (n = 50 cells from each of three independent experiments).

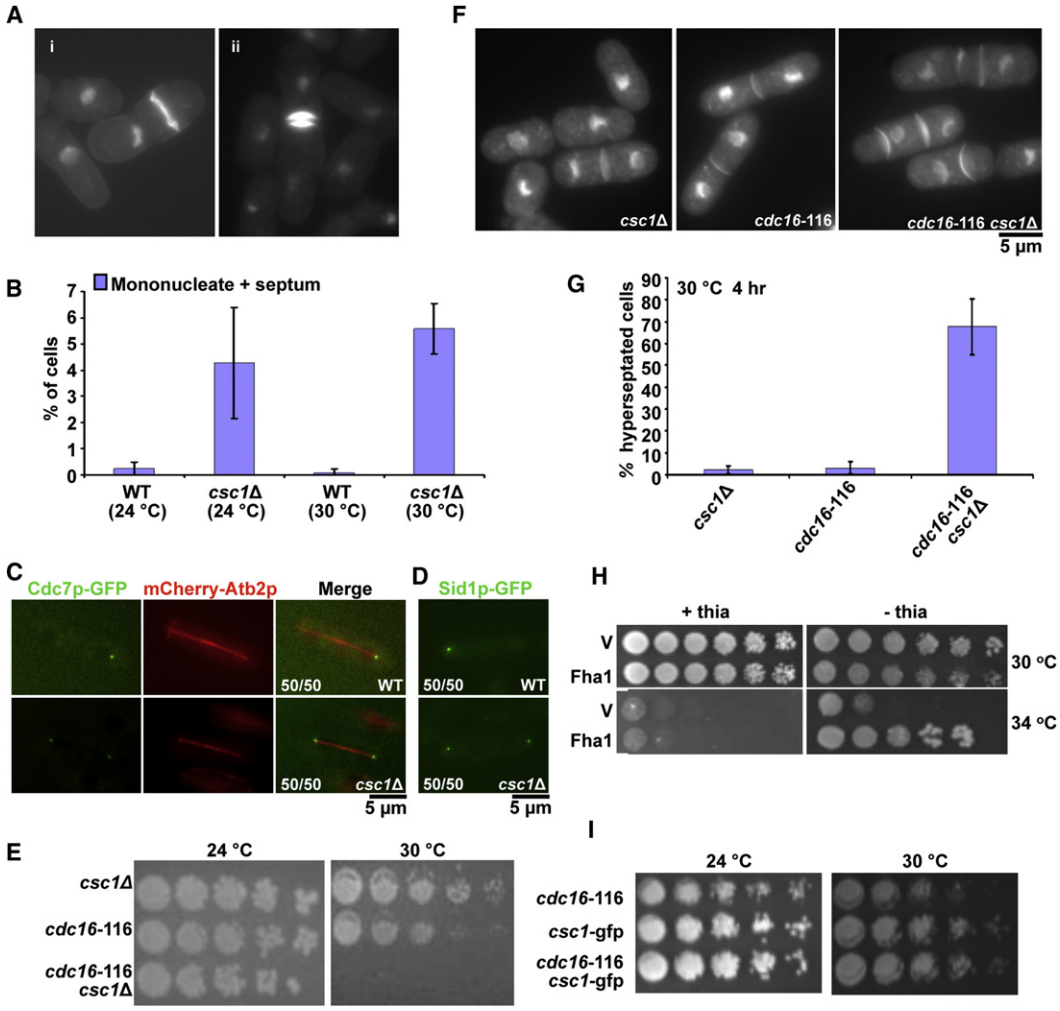

Figure 2. Csc1p Promotes Asymmetric Localization of SIN Kinases Cdc7p and Sid1p

(A) *csc1⊿* generates uninucleate cells with a division septum and binucleate cells with more than one septum. *csc1⊿* cells were grown at 30°C, fixed, and stained with DAPI (for DNA) and aniline blue (for cell wall).

(B) Loss of Csc1p function can cause inappropriate septation in interphase. Wild-type (WT) and *csc1⊿* cells were grown at 24°C or 30°C, treated with 12 mM hydroxyurea for 6 hr to arrest cells at S phase. Interphase cells with a division septum were quantitated (n = 500 cells from each of three independent experiments). Cells with septum deposition were counted for WT as well as *csc1⊿*. Error bars in (B) and (G) represent standard deviations.

(C and D) Cdc7p and Sid1p localize symmetrically in *csc1⊿* cells. Wild-type cells expressing Cdc7p or Sid1p-GFP and mCherry-Atb2p were fixed with methanol, and the localization of the fluorescent proteins was investigated. The fraction of cells displaying Cdc7p or Sid1p in an asymmetric configuration or the fraction of *csc1*Δ cells displaying a symmetric distribution of Cdc7p or Sid1p is indicated.

(E) Simultaneous compromise of Csc1p and Cdc16p function leads to growth defects. *csc1⊿*, *cdc16*-116, and *csc1⊿ cdc16*-116 cultures were serially diluted 5-fold four times, spotted on YES agar medium, and grown at 24°C and 30°C. The two parental strains were capable of colony formation at 24°C and 30°C. The *csc1⊿ cdc16*-116 double mutant was capable of colony formation at 24°C, but not at 30°C.

(F) Simultaneous compromise of Csc1p and Cdc16p generates cells with multiple septa. *csc1⊿*, *cdc16*-116, and *csc1⊿ cdc16*-116 mutants were grown at 24°C until early log phase, shifted to 30°C for 3 hr, fixed, and stained with DAPI and aniline blue.

(G) Graphical representation of the percentage of hyperseptated cells in strains described in (F) (n = 500 cells from each of three independent experiments).

(H) Rescue of *cdc16*-116 growth defect with Csc1p overexpression (v-pREP41-*GST* and csc1-pREP41-*csc1-GST*).

(I) Csc1p-GFP cells do not display synthetic lethality with *cdc16*-116. *csc1-GFP*, *cdc16*-116, and *csc1-GFP cdc16*-116 cultures were serially diluted 5-fold, spotted on YES agar plates, and incubated at 24°C and 30°C.

Csc1p might participate in inhibiting the SIN. Previous studies have shown that the inactivation of SIN inhibitors leads to septation even in interphase [21, 24, 27]. Consistent with Csc1p participating as an inhibitor of the SIN, 4%–6% of S phase-arrested *csc1*Δ cells contained a division septum (Figure 2B). The lower percentage of uninucleate cells with a division septum suggested that Csc1p participates in, but is not essential for, SIN inactivation.

(F) Csc1p-GFP asymmetry is observed in wild-type cells. Wild-type cells expressing Csc1p-GFP and mCherry-Atb2p were grown at 24°C until mid-log phase, fixed with methanol, and scored for the symmetric or asymmetric distribution of Csc1p in cells with short spindles (n = 50 cells from each of three independent experiments).

(G) *cdc25*-22 cells expressing Csc1p-GFP and Cdc7p-mCherry were grown at the permissive temperature and imaged by time-lapse microscopy. Elapsed time (in minutes) after the start of imaging is indicated above the panels. Arrows indicate positions where asymmetry of Cdc7p and Csc1p is apparent.

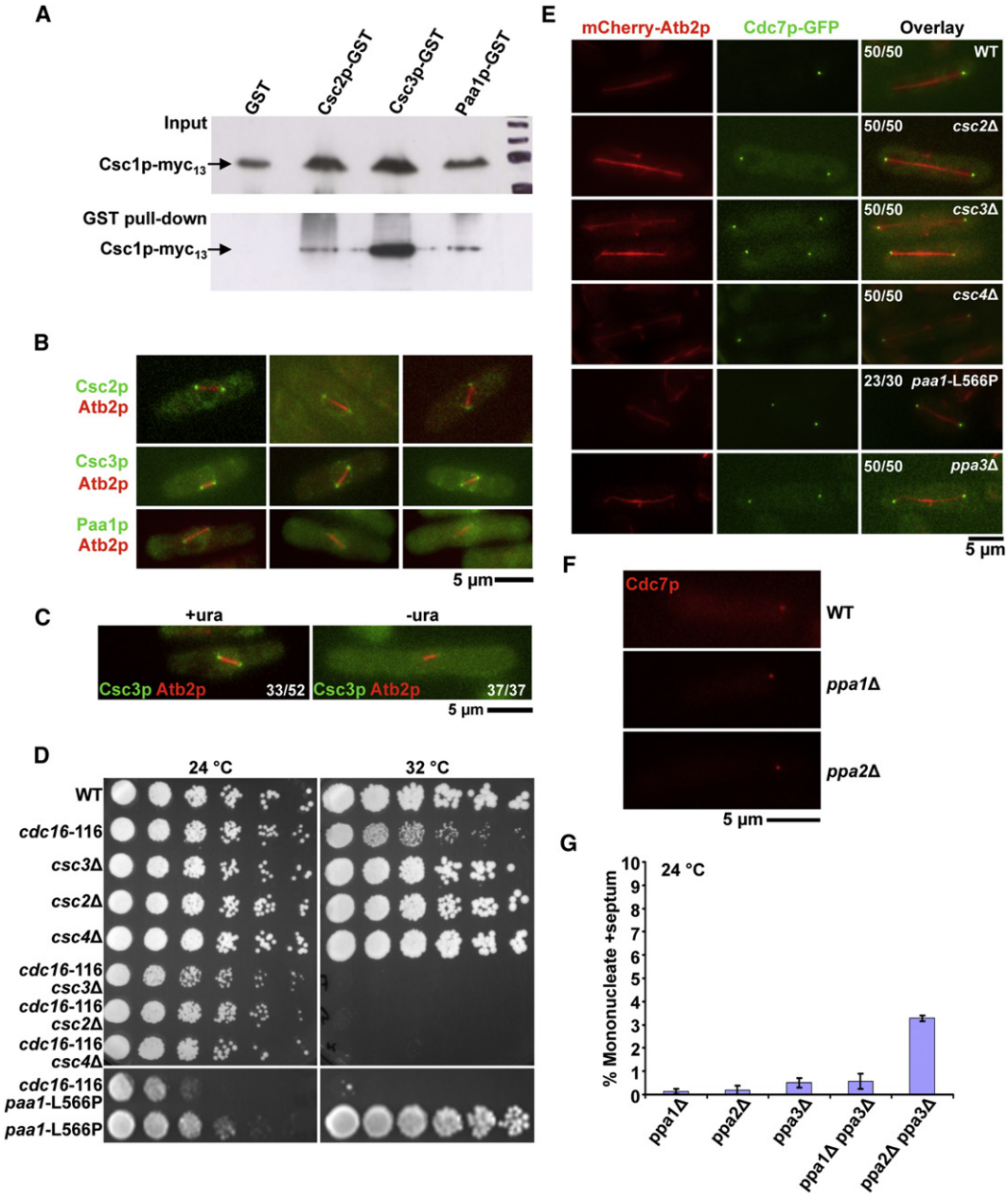

Figure 3. Identification of a Multiprotein Complex Required for Generation of Cdc7p Asymmetry

(A) Csc2p, Csc3p, and Paa1p bind Csc1p-Myc$_{13}$. GST, Csc3-GST, Csc2-GST, and Paa1-GST were isolated from yeast cells expressing Csc1p and one of these proteins using glutathione-linked agarose beads. The isolated proteins were immunoblotted with an antibody against the Myc epitope. The top panel shows total lysates probed with the anti-Myc antibody, whereas the bottom panel shows an immunoblot of the proteins isolated from glutathione agarose beads and probed with the anti-Myc antibody.

(B) Csc2p, Csc3p, and Paa1p localize to early mitotic SPBs. Cells individually expressing mCherry-Atb2p and one of the fusion proteins Csc2p-GFP, Csc3p-GFP, or Paa1p-GFP were grown at 24°C, and the localization patterns of the fluorescent proteins were investigated. Shown are three examples in each case of cells with short mitotic spindles.

(C) The SPB component Ppc89p is required for Csc3p localization to the SPB. Spores from a *ppc89::ura4*$^+$/*ppc89*$^+$ strain expressing Csc3p-GFP were germinated in minimal media in the presence or absence of uracil. The cells were then observed for the localization of Csc3p during early mitosis. The proportion of spores germinated in medium containing uracil and exhibiting a short spindle and Csc3p on SPBs is indicated. In addition, the proportion of spores germinated in the absence of uracil and exhibiting a short spindle and Csc3p on the SPB is indicated.

(D) *csc2Δ*, *csc3Δ*, *csc4Δ*, and *paa1*-L566P display synthetic-lethal interactions with *cdc16*-116. Serial dilutions of *csc2Δ cdc16*-116, *csc3Δ cdc16*-116, *csc4Δ cdc16*-116, and *paa1*-L566P *cdc16*-116 and the corresponding single mutants were spotted on YES agar medium and incubated at 24°C or at 30°C.

(E) Csc2p, Csc3p, Csc4p, Paa1p, and Ppa3p are required for the generation and maintenance of Cdc7p asymmetry. *csc2Δ*, *csc3Δ*, *csc4Δ*, and *ppa3Δ* cells expressing Cdc7p-GFP and mCherry-Atb2p were grown at 24°C, fixed with methanol, and imaged for Cdc7p localization during late anaphase. *paa1*-L566P cells expressing Cdc7p-GFP and mCherry-Atb2p were grown at 24°C and then shifted to 36°C, and the localization of Cdc7p during late anaphase was investigated. The proportion of cells exhibiting symmetric localization of Cdc7p in the various mutants and the proportion of wild-type cells showing asymmetric distribution of Cdc7p are indicated.

SIN involves a linear cascade of three kinases—Cdc7p, Sid1p, and Sid2p, in order of their activation [6, 14–16]. Of these, Cdc7p and Sid1p localize only to the new SPB late in anaphase [17, 32]. Constitutive activation of the SIN, by the inactivation of inhibitors of the SIN, leads to localization of Cdc7p and Sid1p to both SPBs in mitotic cells and to the single SPB in interphase cells [17, 32]. Because deletion of *csc1* led to phenotypes consistent with weak SIN activation, we checked the localization of Cdc7p and Sid1p in *csc1*Δ cells (Figures 2C and 2D). Whereas Cdc7p and Sid1p were only detected on one SPB in late anaphase in wild-type cells, Cdc7p and Sid1p were found on both SPBs in 100% of late anaphase *csc1*Δ cells (Figures 2C and 2D). These experiments establish that Csc1p is essential for the asymmetric localization of Cdc7p and Sid1p.

Previous work has shown that inactivation of SIN (using loss of Cdc7p from the SPB as an assay) occurs in coordination with actomyosin ring closure and division of the cytoplasm [28]. Because Cdc7p was detected on both SPBs in *csc1*Δ cells but was not detected on interphase SPBs, we tested whether loss of Cdc7p from the SPBs coincided with full constriction of the actomyosin ring. As in wild-type cells, Cdc7p signals were lost from both the SPBs before or upon completion of actomyosin ring constriction and septum assembly and did not persist after septation (Figure S2, approximately after time point 60′). Curiously, we found that Cdc7p was not lost simultaneously from both SPBs but was lost from one SPB first, followed by its loss from the other. Nevertheless, these experiments further substantiate the view that division of the cytoplasm promotes the removal of Cdc7p from the SPB.

To assess whether Csc1p functions in concert with the two-component GAP (Cdc16p-Byr4p) [21, 33, 42] for Spg1p, we generated double mutants defective in *cdc16* and *csc1*. Whereas each of the single mutants was capable of colony formation at the semipermissive temperature of 30°C, the double mutant was unable to do so (Figure 2E) and died as a result of hyperactivation of the SIN (>80% of cells displaying multiple septa; Figures 2F and 2G). The synthetic genetic interaction suggested that Csc1p function is crucial when the major inhibitors of SIN are even partially compromised.

Previous work has shown that overproduction of many negative regulators of the SIN, such as Byr4p, Zfs1p, and Dma1p, causes rescue of *cdc16*-116 mutants [23, 43, 44]. In light of this, we tested whether overproduction of Csc1p, a new negative regulator of SIN, could suppress *cdc16*-116. A plasmid capable of expressing high levels of Csc1p and a control plasmid were introduced into *cdc16*-116 cells. We noticed that, consistent with the role of Csc1p in inhibition of SIN, overproduction of Csc1p allowed *cdc16*-116 to form colonies under conditions in which the control plasmid-expressing cells were unable to do so (Figure 2H).

The discovery of a phenotype associated with *csc1*Δ allowed us to test whether Csc1p-GFP was a functional version of this protein. Synthetic lethality was not detected in *cdc16*-116 *csc1*-GFP strains (Figure 2I). Moreover, because Cdc7p asymmetry was not compromised in Csc1p-GFP-expressing cells (Figure 1G), we concluded that Csc1p-GFP was fully functional in our assay conditions.

Csc1p Is Present in a Multiprotein Complex Containing a New Catalytic Subunit of Protein Phosphatase 2A, Ppa3p

To gain biochemical insights into Csc1p functions, we carried out an immunoaffinity purification of Csc1p-Myc_{13} (Figure S3), in which four major Coomassie-stained bands were detected. Proteins that associated with Myc_{13} beads were identified by tandem mass spectrometry. In this analysis, we identified four additional proteins, Csc2p (SPBC27B12.04c), Csc3p (SPBC1773.02), Csc4p (SPAC2C4.10c), and the scaffold A subunit of PP2A, Paa1p [45]. Csc2p is most related to the budding yeast Far11p [46], Csc3p is most related to budding yeast Far8p [46], and Csc4p is an uncharacterized protein with no known orthologs. Csc2p contains the DUF3402 domain, whereas Csc3p is related to the conserved striatin family of proteins [47, 48]. To confirm the physical interactions, we incubated lysates extracted from cells expressing Csc1p-Myc_{13} and each of the following proteins: glutathione S-transferase (GST), Csc2p-GST, Csc3p-GST, or Paa1p-GST with glutathione Sepharose. Csc1p was not detected in pull-downs from cells expressing GST alone but was detected in pull-downs from cells expressing Csc2p-GST, Csc3p-GST, and Paa1p-GST (Figure 3A). We purified Csc3p-TAP and Paa1p-TAP and used liquid chromatography-tandem mass spectrometry to identify proteins interacting with these proteins (Table S1). In both cases, Csc1p, Csc2p, and Csc4p were identified. In addition, Csc3p was detected in the Paa1p-TAP complex and Paa1p was detected in the Csc3p-TAP complex. Although a PP2A catalytic subunit was not detected in our original Csc1p purification, a novel catalytic subunit of PP2A (which we have termed Ppa3p; open reading frame SPAC22H10.04) was identified among proteins copurifying with Csc3p-TAP and Paa1p-TAP. These experiments established that Csc1p physically associates with Csc2p, Csc3p, Csc4p, Paa1p, and Ppa3p.

We then investigated the cellular localization of these Csc1p-associated proteins using strains expressing Csc2p, Csc3p, Csc4p, Paa1p, and Ppa3p as GFP fusions under the control of endogenous regulatory sequences. Signals of Ppa3p and Csc4p were undetectable (data not shown). However, we were able to visualize the other four proteins. Csc2p and Csc3p, like Csc1p, were detected in early mitotic cells on both SPBs (Figure 3B). In addition, Csc2p and Csc3p were also detected around the nuclear envelope (Figure 3B). In approximately 25%–30% of cells with short spindles, Csc2p and Csc3p segregated asymmetrically to the SPB containing less Cdc7p (Figure S4), and in approximately 40%–50% of cells, Csc2p and Csc3p signals were of equal intensity on both SPBs (Figure S4). We were unable to ascertain the fate of Csc2p and Csc3p in the rest of the cells as a result of the strong nuclear envelope fluorescence of Csc2p and Csc3p (data not shown). Paa1p was weakly detected on mitotic SPBs and was also detected at the actomyosin ring late in mitosis (Figure 3B). The localization experiments therefore established that Csc2p, Csc3p, and Paa1p colocalize

(F) Cdc7p asymmetry is not compromised in the absence of Ppa1p and Ppa2p. Wild-type, *ppa1*Δ, and *ppa2*Δ cells expressing mCherry-Cdc7p were grown at 24°C, and the localization of Cdc7p during late anaphase was investigated.

(G) Combined loss of Ppa2p and Ppa3p leads to a modest increase in uninucleate cells with division septum. *ppa1*Δ, *ppa2*Δ, *ppa3*Δ, *ppa1*Δ *ppa3*Δ, and *ppa2*Δ *ppa3*Δ cells were cultured in the presence of 12 mM hydroxyurea, and the proportion of uninucleate cells with a division septum was estimated (n = 500 cells from each of three independent experiments). Error bars represent standard deviations.

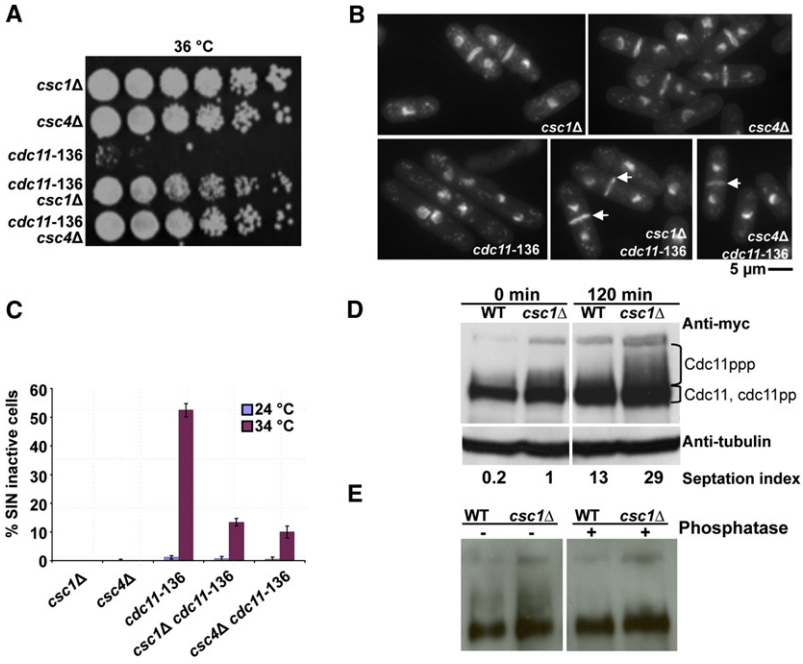

Figure 4. SIP Complex Interacts Physically with the SIN Scaffold Protein Cdc11p and Promotes Dephosphorylation of Cdc11p

(A) Restoration of colony formation ability of *cdc11*-136 upon deletion of *csc1* or *csc4* genes. Cultures of *csc1*Δ, *csc4*Δ, *cdc11*-136, *csc1*Δ *cdc11*-136, and *csc4*Δ *cdc11*-136 grown overnight at 24°C were serially diluted 5-fold five times and spotted on YES agar plates and incubated at 36°C.

(B) *cdc11*-136 *csc1*Δ and *cdc11*-136 *csc4*Δ cells assemble normal-appearing septa. *csc1*Δ, *csc4*Δ, *cdc11*-136, *csc1*Δ *cdc11*-136, and *csc4*Δ *cdc11*-136 cells were grown at 24°C and shifted to 36°C. The cells were fixed and stained with aniline blue (cell wall) and DAPI (DNA).

(C) The percentages of multinucleate cells in all strains described in (B) were estimated and plotted to gain a measure of the extent of suppression of *cdc11*-136 by loss of function of *csc1* or *csc4* (n = 500 cells from each of three independent experiments). Error bars represent standard deviations.

(D) Cdc11p extracted from *csc1⊿* cells migrates slower than Cdc11p from wild-type cells. *cdc11-myc*$_{13}$ and *cdc11-myc*$_{13}$ *csc1⊿* were arrested at S phase using 12 mM hydroxyurea for 4 hr. The cells were released by washing three times with YES, and samples were collected at indicated times. Cell extracts were resolved on 8% SDS-PAGE, and immunoblot analysis was carried out using an anti-Myc antibody.

(E) SIP promotes dephosphorylation of Cdc11p. *nda3*-KM311 *cdc11-myc*$_{13}$ and *nda3*-KM311 *cdc11-myc*$_{13}$ *csc1*Δ cells were arrested at prometaphase by shift to 18°C for 4 hr and released to 32°C. The phosphorylation state of Cdc11p was then analyzed after 50 min of release by immunoblot analysis using anti-Myc antibody. Cell lysates were prepared and immunoblotted with antibodies against the Myc epitope (left panel). To establish that the slower migration observed in *csc1*Δ cells was a result of increased phosphorylation of Cdc11p, lysates were treated with lambda phosphatase (right panel).

significantly during early mitosis and that Csc2p and Csc3p exhibit asymmetric segregation to the SPB containing less Cdc7p.

We have shown that Csc1p requires Ppc89p to localize to the SPB (Figure 1C). We then tested whether other Csc1p interactors also depend on Ppc89p to localize to the SPB. To this end, we investigated the localization of Csc3p in *ppc89* mutants. Csc3p localized to the SPB in 33 of 52 mitotic cells (Figure 3C) from the diploid *ppc89*Δ/*ppc89*$^{+}$ upon spore germination in medium containing uracil (which allows germination of *ppc89*$^{+}$ and *ppc89*Δ spores). However, Csc3p failed to localize to the SPB in 37 of 37 cases (Figure 3C) when spores were germinated in medium lacking uracil (which selectively allows germination of *ppc89*Δ spores). Thus Csc3p, like Csc1p, requires functional Ppc89p to localize to the SPB.

Next, we tested whether Csc2p, Csc3p, Csc4p, Paa1p, and Ppa3p function as inhibitors of the SIN. Toward this goal, we generated strains deleted for the *csc2*, *csc3*, *csc4*, and *ppa3* genes. All four genes were found to be dispensable for cell growth. Because Paa1p is essential for cell viability [45], we generated a temperature-sensitive mutant, *paa1*-L566P, which was capable of colony formation at 24°C and weakly capable at 32°C but died at 36°C. As in the case of *csc1*Δ *cdc16*-116 double mutants, double mutants of the genotypes *cdc16*-116 *csc2*Δ, *cdc16*-116 *csc3*Δ, *cdc16*-116 *csc4*Δ, and *cdc16*-116 *paa1*-L566P were all unable to grow and form colonies under conditions in which the parental single mutants were capable of colony formation (Figure 3D). Furthermore, as in *csc1*Δ cells, Cdc7p localized to both SPBs in 100% of late mitotic *csc2*Δ, *csc3*Δ, *csc4*Δ, *paa1*-L566P, and *ppa3*Δ (Figure 3E). Double mutants (in all possible pairwise combinations) defective in two of the six members of the SIP were generated. These double mutants did not display significant additive effects (Table S2). In addition, loss of function of any of these genes led to a failure of localization of each of the members of the multiprotein complex to the SPB (Table S3). Collectively, the biochemical, genetic, and cytological analyses establish that Csc1p functions in a PP2A-associated multiprotein complex containing at least five other proteins—Csc2p, Csc3p, Csc4p, Paa1p, and Ppa3p—which functions as a weak inhibitor of the SIN and promotes SIN asymmetry. We refer to this complex as the SIN-inhibitory PP2A complex (SIP).

Having identified Ppa3p, a new PP2A catalytic subunit, as a member of the SIP, we tested whether the known catalytic subunits Ppa1p and Ppa2p also play a role in the generation of Cdc7p asymmetry. As in wild-type cells, Cdc7p localized to only one SPB in *ppa1*Δ and *ppa2*Δ mutants (Figure 3F), suggesting that Ppa1p and Ppa2p do not directly participate in the generation of Cdc7p asymmetry. However, it is possible that Ppa2p might play a minor role in SIN inactivation, because *ppa2*Δ *ppa3*Δ double mutants, but not *ppa1*Δ *ppa3*Δ double mutants, showed a modest increase in the number of septated interphase cells (Figure 3G).

SIP Promotes Dephosphorylation of Cdc11p

In the course of experiments aimed at studying potential genetic interactions between SIP mutants and SIN mutants, we found that the inability of *cdc11*-136 [49] to form colonies at 36°C was reversed by the introduction of the *csc1⊿* mutation (Figure 4A). Unlike in the *cdc11*-136 single mutants, septa of normal appearance were detected in *cdc11*-136 *csc1*Δ (Figure 4B). Furthermore, the percentage of cells with either multiple nuclei or two clustered nuclei (denoted "SIN inactive" in Figure 4C) were dramatically reduced in *cdc11*-136 *csc1*Δ (Figure 4C). A similar suppression of the temperature-sensitive lethality of *cdc11*-136 was also observed in *cdc11*-136 *csc4*Δ double mutants (Figures 4A–4C). Furthermore, *csc2* and *csc3*

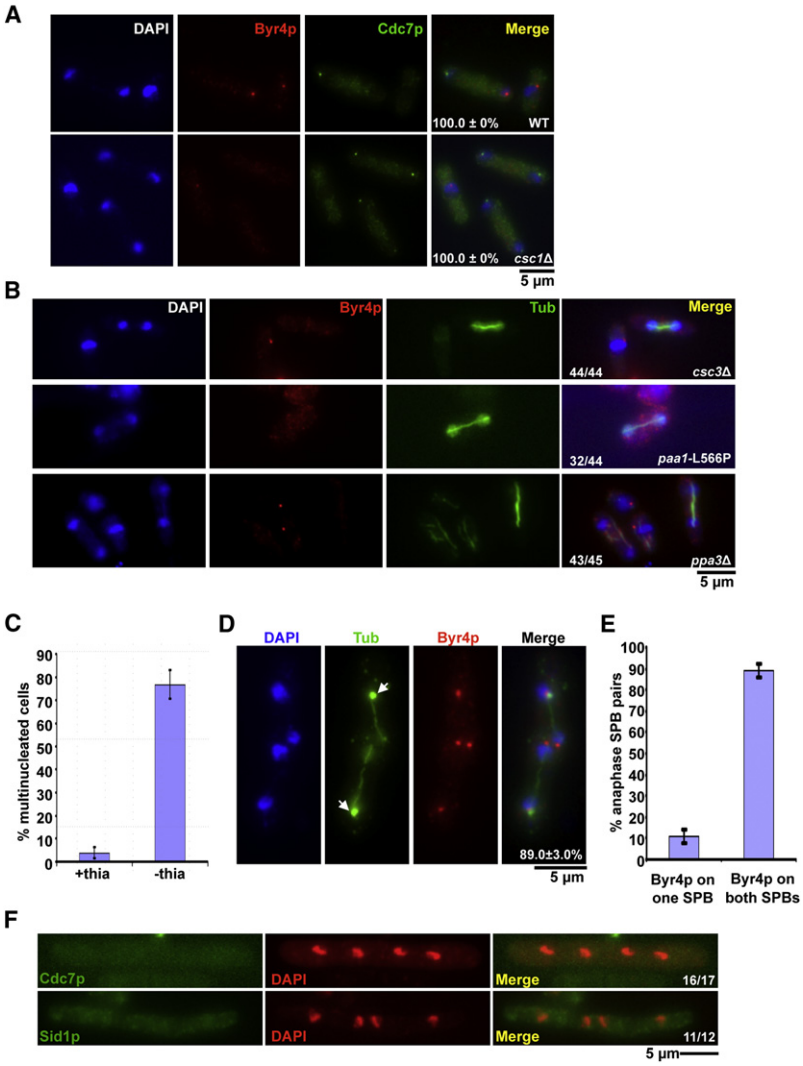

Figure 5. Modulation of SIP Affects the Localization of SIN and Its Regulators

(A and B) Byr4p localization to the mitotic SPB depends on SIP.

(A) Wild-type and *csc1*Δ cells expressing Cdc7p-3×HA were stained using antibodies against the HA epitope and against Byr4p. After staining, cells were mounted in DAPI and images were acquired (n = 50 cells from each of three independent experiments).

(B) *csc3*Δ and *ppa3*Δ cells grown at 24°C and *paa1*-L566P cells grown at 36°C were stained with antibodies against tubulin and Byr4p and mounted in DAPI medium, and images were acquired.

(C) Overexpression of Csc1p leads to a failure in cytokinesis. Csc1p was overexpressed by growing *nmt1-csc1-GFP* cells in minimal medium lacking thiamine for 16 hr at 24°C and fixing and staining them with aniline blue (cell wall) and DAPI (DNA). The percentage of multinucleated cells was then determined (n = 500 cells from each of three independent experiments). Error bars in (C) and (E) represent standard deviations.

(D) Overproduction of Csc1p leads to recruitment of Byr4p to both SPBs in mitotic cells. *nmt1-csc1-GFP* cells were grown and fixed as in (C). The cells were then stained using antibodies against Byr4p and tubulin TAT-1 antibody. Arrows indicate increased accumulation of GFP-Csc1p (n = 50 cells from each of three independent experiments).

(E) Quantitation of the percentage of anaphase SPB pairs with Byr4p on one or both SPBs (n = 50 cells from each of three independent experiments).

(F) Cdc7p and Sid1p are displaced from mitotic SPBs upon Csc1p overexpression. *nmt1-csc1-GFP* expressing Cdc7p-mCherry were grown in EMM medium lacking thiamine for 16 hr at 24°C, fixed with methanol, and imaged. For Sid1p visualization, *nmt1-csc1-GFP* cells expressing Sid1p-Myc_{13} were stained with antibodies against the Myc epitope. The fraction of late anaphase cells in which Cdc7p or Sid1p was not detected on SPBs is indicated.

mutants also suppressed *cdc11*-136 mutants, although the efficiency was reduced compared to the suppression by *csc1* and *csc4* (data not shown). These observations suggested that SIP might function in the generation of Cdc7p asymmetry by modulating Cdc11p function.

Previous work has shown that Cdc11p becomes phosphorylated during progression through mitosis and that PP2A is required for Cdc11p dephosphorylation [50]. We therefore considered the possibility that SIP might promote dephosphorylation of Cdc11p, thereby negatively regulating SIN. To address this possibility, we generated hydroxyurea-synchronized cultures of wild-type and *csc1*Δ. As shown by other investigators, a slower-migrating smear of Cdc11p appeared with progression through mitosis in wild-type cells (Figure 4D). Importantly, the intensity of the slower-migrating smear of Cdc11p was increased in *csc1*Δ cells (Figure 4D). We also confirmed decreased mobility and increased smear of Cdc11p in *csc1*Δ cells synchronized via a metaphase block-and-release using the *nda3*-KM311 mutation. Because the smearing was lost upon treatment with phosphatase (Figure 4E), we concluded that the smear pattern was a result of increased phosphorylation of Cdc11p and that Csc1p (and the SIP) participates in the dephosphorylation of Cdc11p.

Byr4p Localization to the SPB Is Compromised in SIP-Complex Mutants

Byr4p, an essential component of the Spg1p GAP complex, has been shown to bind to dephosphorylated Cdc11p [50]. Because the SIP concentrates on the SPB lacking Cdc7p, we hypothesized that a potential function of SIP might be to dephosphorylate Cdc11p, thereby promoting Byr4p relocalization to the SPB containing the increased amount of SIP. If this were the case, Byr4p should not be detected on either SPB during anaphase in SIP mutants. To address this issue, we fixed and stained wild-type and *csc1*Δ cells expressing Cdc7p-3×HA with antibodies against HA and Byr4p. In late mitotic wild-type cells, Cdc7p and Byr4p were detected on opposite SPBs (Figure 5A). By contrast, in *csc1*Δ cells Cdc7p was detected at both anaphase SPBs, and importantly, Byr4p was not detected on either SPB in mitotic cells (Figure 5A). Byr4p was also undetectable at the SPB in *csc3*Δ, *paa1*-L566P, and *ppa3*Δ cells (Figure 5B). In wild-type cells, Byr4p localizes to SPBs during interphase [50]. Curiously, Byr4p was readily detected on the SPB in uninucleate (interphase) wild-type, *csc1*Δ, *csc3*Δ, *paa1*-L566P, and *ppa3*Δ cells (see interphase cells in Figures 5A and 5B), suggesting that the regulation of Byr4p localization by the SIP is specific to mitosis.

If SIP plays a role in recruiting Byr4p to the SPB, it would be expected that overproduction of Csc1p and/or other members of the SIP might cause inappropriate recruitment of Byr4p to the SPB, leading to failure of cytokinesis. To this end, Csc1p-GFP was overexpressed in wild-type cells. Under inducing conditions, nearly 75% of the cells became multinucleated (Figure 5C). Furthermore, unlike in wild-type cells, Byr4p was recruited to each of the two SPBs connecting elongated anaphase B spindles in more than 85% of the cells with two mitotic spindles (Figures 5D and 5E). Conversely, in Csc1p-overexpressing cells, Cdc7p and Sid1p failed to localize to any of the SPBs in mitotic cells with four nuclei (Figure 5F). Sid2p localization was unaltered upon Csc1p overproduction (data not shown). We conclude that excess Csc1p promotes inappropriate retention of Byr4p at SPBs into anaphase and failure of retention of Cdc7p and Sid1p at the SPBs.

Discussion

In this study, we have identified a SIN-inhibitory PP2A complex that we have termed SIP. SIP contains at least six proteins in unknown relative stoichiometry, including a new catalytic subunit of PP2A (Ppa3p); the A subunit of PP2A (Paa1p) [45]; three proteins related to the budding yeast proteins Far8p (Csc3p), Far10p (Csc1p), and Far11p (Csc2p) [46]; and a novel protein, Csc4p. Four of the six components of the SIP localize to the SPB; we were unable to determine the localization patterns of the other two through introduction of a C-terminal GFP tag. Interestingly, Csc1p and Csc3p depended on Ppc89p rather than the known SIN scaffolds Cdc11p and Sid4p to localize to the SPB. As expected for a protein complex, the SPB localization of each SIP component depended on all other SIP components, and double SIP mutants displayed phenotypes similar to single mutants, suggesting that the SIP functions as a single unit to regulate the SIN.

SIP localizes to both SPBs in early mitosis and then transiently asymmetrically localizes to the old SPB that would contain Byr4p (i.e., the SPB that loses Cdc7p and Sid1p), before disappearing from both SPBs prior to full elongation of the mitotic spindle. These observations suggest a role for SIP in the regulation of SIN asymmetry or function. We found that loss of SIP function led to a weak SIN-activated phenotype, in that binucleate cells with two or more septa or uninucleate cells with septa were detected. Based on these observations, we conclude that SIP functions as a negative regulator of the SIN, consistent with previous genetic studies demonstrating suppression of SIN mutants by other PP2A mutants [51–53].

Loss of SIP function leads to symmetric localization of Cdc7p and Sid1p to both SPBs in 100% of mitotic cells. Despite the presence of Cdc7p and Sid1p on both SPBs in mitotic cells defective in SIP function, mutants defective in SIP are viable, unlike *cdc16*-116 and *byr4*Δ mutants [21, 24], in which Cdc7p has also been shown to localize to both SPBs. The fact that Cdc7p leaves both SPBs upon or before completion of septation in SIP mutants and the presence of a mechanism to prevent localization of Cdc7p to the SPBs in interphase SIP mutant cells might account for the viability of SIP mutants. It is likely that localization of Cdc7p and Sid1p to both SPBs may only activate SIN moderately over the wild-type levels (as gauged by a modest but reproducible increase in septated cells). By contrast, continued maintenance of Cdc7p and Sid1p on the SPB following completion of septation and in subsequent interphase might lead to hyperactivation of the SIN and uncontrolled septation.

Byr4p fails to localize to the SPB during mitosis in SIP mutants. However, Byr4p localization to the SPB during interphase was not compromised in SIP mutants. These observations indicate that distinct mechanisms regulate the SPB localization of Byr4p during these different cell-cycle stages. We have shown that overproduction of Csc1p leads to cytokinesis defects akin to those observed in SIN mutants, in that Cdc7p and Sid1p fail to localize to mitotic SPBs. It is possible that Csc1p might function as a key member of the SIP, whose presence at the SPB might lead to recruitment of other members of the SIP to the SPB.

How does SIP regulate Cdc7p (and Sid1p-Cdc14p) asymmetry? Our studies, in conjunction with studies of Krapp and colleagues [50], provide a molecular framework for the first steps in the establishment and propagation of asymmetry in Cdc7p during mitosis and cytokinesis, and a mechanistic explanation for the ability of some PP2A mutants to suppress SIN mutants [51–53]. It is likely that the establishment and maintenance of an asymmetric state of Cdc7p (and Sid1p localization) [15, 32] might operate in multiple steps, with the innate asymmetry of the age of the spindle pole body [31] potentially playing an important role. We believe that entry into mitosis and CDK activation lead to the symmetric localization of Cdc7p [32] and the SIP. It is possible that the SPB scaffold Cdc11p might be more accessible for dephosphorylation by the SIP at the old SPB. Because Byr4p binds preferentially to dephosphorylated Cdc11p [50], it is likely that the decreased phosphorylation of Cdc11p might lead to further recruitment of Byr4p from the newer SPB to the older SPB. The fact that Cdc11p phosphorylation is increased in *par1* [50] and *csc1* mutants suggests that SIP (via PP2A catalytic activity) dephosphorylates Cdc11p, leading to loss of Cdc7p from the old SPB and the recruitment of Byr4p to it. Our finding that Byr4p fails to localize to either of the SPBs in *csc1*Δ, *csc3*Δ, *paa1*-L566P, and *ppa3*Δ cells and that Byr4p localizes symmetrically in late mitotic cells overexpressing Csc1p is consistent with this view. As Cdc7p departs from the old SPB, further phosphorylation of Cdc11p on the new SPB might further reinforce Cdc7p at the new SPB, leading to a stable asymmetric state. A key question for the future is how the age of the SPB promotes and/or inhibits phosphorylation events that are key to symmetry breaking.

Proteins related to 5 of 6 SIP members are conserved across evolution and are found from yeasts to human. A SIN-like network (the MEN) is also known to exist in budding yeast [3, 54]. Because members of the MEN localize asymmetrically to one of the two mitotic SPBs, future studies should test whether SIP-like proteins participate in the generation or propagation of MEN asymmetry in budding yeast. Interestingly, in animal cells, a protein complex termed the STRIPAK [55] (related to the SIP) has been shown to negatively regulate the Hippo-Lats pathway [10] (members of which are related to those of the SIN), although the mechanism of this negative regulation is not fully understood. Thus, the mechanism of SIP function that we describe in this study might be applicable to that of related protein complexes in other organisms.

Supplemental Information

Supplemental Information includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.10.051.

Acknowledgments

Many thanks are due to D. McCollum and M. Sato for yeast strains. We wish to thank members of the Balasubramanian laboratory for helpful suggestions and S. Oliferenko, M. Mishra, R. Srinivasan, and D. Subramaniam for critical reading of the manuscript. This work was funded by the Singapore Millennium Foundation (M.K.B.) and the Howard Hughes Medical Institute, of which K.L.G. is an investigator.

Received: February 9, 2011
Revised: October 13, 2011
Accepted: October 28, 2011
Published online: November 23, 2011

References

1. Eggert, U.S., Mitchison, T.J., and Field, C.M. (2006). Animal cytokinesis: from parts list to mechanisms. Annu. Rev. Biochem. *75*, 543–566.
2. Pollard, T.D., and Wu, J.Q. (2010). Understanding cytokinesis: lessons from fission yeast. Nat. Rev. Mol. Cell Biol. *11*, 149–155.
3. Balasubramanian, M.K., Bi, E., and Glotzer, M. (2004). Comparative analysis of cytokinesis in budding yeast, fission yeast and animal cells. Curr. Biol. *14*, R806–R818.
4. Gould, K.L., and Simanis, V. (1997). The control of septum formation in fission yeast. Genes Dev. *11*, 2939–2951.
5. Goyal, A., Takaine, M., Simanis, V., and Nakano, K. (2011). Dividing the spoils of growth and the cell cycle: The fission yeast as a model for the study of cytokinesis. Cytoskeleton (Hoboken) *68*, 69–88.
6. McCollum, D., and Gould, K.L. (2001). Timing is everything: regulation of mitotic exit and cytokinesis by the MEN and SIN. Trends Cell Biol. *11*, 89–95.
7. Krapp, A., and Simanis, V. (2008). An overview of the fission yeast septation initiation network (SIN). Biochem. Soc. Trans. *36*, 411–415.
8. Guertin, D.A., Trautmann, S., and McCollum, D. (2002). Cytokinesis in eukaryotes. Microbiol. Mol. Biol. Rev. *66*, 155–178.
9. Hou, M.C., Salek, J., and McCollum, D. (2000). Mob1p interacts with the Sid2p kinase and is required for cytokinesis in fission yeast. Curr. Biol. *10*, 619–622.
10. Ribeiro, P.S., Josué, F., Wepf, A., Wehr, M.C., Rinner, O., Kelly, G., Tapon, N., and Gstaiger, M. (2010). Combined functional genomic and proteomic approaches identify a PP2A complex as a negative regulator of Hippo signaling. Mol. Cell *39*, 521–534.
11. Tomlin, G.C., Morrell, J.L., and Gould, K.L. (2002). The spindle pole body protein Cdc11p links Sid4p to the fission yeast septation initiation network. Mol. Biol. Cell *13*, 1203–1214.
12. Chang, L., and Gould, K.L. (2000). Sid4p is required to localize components of the septation initiation pathway to the spindle pole body in fission yeast. Proc. Natl. Acad. Sci. USA *97*, 5249–5254.
13. Mehta, S., and Gould, K.L. (2006). Identification of functional domains within the septation initiation network kinase, Cdc7. J. Biol. Chem. *281*, 9935–9941.
14. Fankhauser, C., and Simanis, V. (1994). The cdc7 protein kinase is a dosage dependent regulator of septum formation in fission yeast. EMBO J. *13*, 3011–3019.
15. Guertin, D.A., Chang, L., Irshad, F., Gould, K.L., and McCollum, D. (2000). The role of the sid1p kinase and cdc14p in regulating the onset of cytokinesis in fission yeast. EMBO J. *19*, 1803–1815.
16. Sparks, C.A., Morphew, M., and McCollum, D. (1999). Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. J. Cell Biol. *146*, 777–790.
17. Guertin, D.A., and McCollum, D. (2001). Interaction between the noncatalytic region of Sid1p kinase and Cdc14p is required for full catalytic activity and localization of Sid1p. J. Biol. Chem. *276*, 28185–28189.
18. Schmidt, S., Sohrmann, M., Hofmann, K., Woollard, A., and Simanis, V. (1997). The Spg1p GTPase is an essential, dosage-dependent inducer of septum formation in Schizosaccharomyces pombe. Genes Dev. *11*, 1519–1534.
19. Krapp, A., Schmidt, S., Cano, E., and Simanis, V. (2001). S. pombe cdc11p, together with sid4p, provides an anchor for septation initiation network proteins on the spindle pole body. Curr. Biol. *11*, 1559–1568.
20. Salimova, E., Sohrmann, M., Fournier, N., and Simanis, V. (2000). The S. pombe orthologue of the S. cerevisiae mob1 gene is essential and functions in signalling the onset of septum formation. J. Cell Sci. *113*, 1695–1704.
21. Minet, M., Nurse, P., Thuriaux, P., and Mitchison, J.M. (1979). Uncontrolled septation in a cell division cycle mutant of the fission yeast Schizosaccharomyces pombe. J. Bacteriol. *137*, 440–446.
22. Fankhauser, C., Marks, J., Reymond, A., and Simanis, V. (1993). The S. pombe cdc16 gene is required both for maintenance of p34cdc2 kinase activity and regulation of septum formation: a link between mitosis and cytokinesis? EMBO J. *12*, 2697–2704.
23. Furge, K.A., Wong, K., Armstrong, J., Balasubramanian, M., and Albright, C.F. (1998). Byr4 and Cdc16 form a two-component GTPase-activating protein for the Spg1 GTPase that controls septation in fission yeast. Curr. Biol. *8*, 947–954.
24. Song, K., Mach, K.E., Chen, C.Y., Reynolds, T., and Albright, C.F. (1996). A novel suppressor of ras1 in fission yeast, byr4, is a dosage-dependent inhibitor of cytokinesis. J. Cell Biol. *133*, 1307–1319.
25. Li, C., Furge, K.A., Cheng, Q.C., and Albright, C.F. (2000). Byr4 localizes to spindle-pole bodies in a cell cycle-regulated manner to control Cdc7 localization and septation in fission yeast. J. Biol. Chem. *275*, 14381–14387.
26. Chang, L., Morrell, J.L., Feoktistova, A., and Gould, K.L. (2001). Study of cyclin proteolysis in anaphase-promoting complex (APC) mutant cells reveals the requirement for APC function in the final steps of the fission yeast septation initiation network. Mol. Cell. Biol. *21*, 6681–6694.
27. Krapp, A., Collin, P., Cano Del Rosario, E., and Simanis, V. (2008). Homoeostasis between the GTPase Spg1p and its GAP in the regulation of cytokinesis in S. pombe. J. Cell Sci. *121*, 601–608.
28. García-Cortés, J.C., and McCollum, D. (2009). Proper timing of cytokinesis is regulated by Schizosaccharomyces pombe Etd1. J. Cell Biol. *186*, 739–753.
29. Guertin, D.A., Venkatram, S., Gould, K.L., and McCollum, D. (2002). Dma1 prevents mitotic exit and cytokinesis by inhibiting the septation initiation network (SIN). Dev. Cell *3*, 779–790.
30. Morrell, J.L., Tomlin, G.C., Rajagopalan, S., Venkatram, S., Feoktistova, A.S., Tasto, J.J., Mehta, S., Jennings, J.L., Link, A., Balasubramanian, M.K., and Gould, K.L. (2004). Sid4p-Cdc11p assembles the septation initiation network and its regulators at the S. pombe SPB. Curr. Biol. *14*, 579–584.
31. Grallert, A., Krapp, A., Bagley, S., Simanis, V., and Hagan, I.M. (2004). Recruitment of NIMA kinase shows that maturation of the S. pombe spindle-pole body occurs over consecutive cell cycles and reveals a role for NIMA in modulating SIN activity. Genes Dev. *18*, 1007–1021.
32. Sohrmann, M., Schmidt, S., Hagan, I., and Simanis, V. (1998). Asymmetric segregation on spindle poles of the Schizosaccharomyces pombe septum-inducing protein kinase Cdc7p. Genes Dev. *12*, 84–94.
33. Cerutti, L., and Simanis, V. (1999). Asymmetry of the spindle pole bodies and spg1p GAP segregation during mitosis in fission yeast. J. Cell Sci. *112*, 2313–2321.
34. Simanis, V. (2003). Events at the end of mitosis in the budding and fission yeasts. J. Cell Sci. *116*, 4263–4275.
35. Krapp, A., Gulli, M.P., and Simanis, V. (2004). SIN and the art of splitting the fission yeast cell. Curr. Biol. *14*, R722–R730.
36. Matsuyama, A., Arai, R., Yashiroda, Y., Shirai, A., Kamata, A., Sekido, S., Kobayashi, Y., Hashimoto, A., Hamamoto, M., Hiraoka, Y., et al. (2006). ORFeome cloning and global analysis of protein localization in the fission yeast Schizosaccharomyces pombe. Nat. Biotechnol. *24*, 841–847.
37. Russell, P., and Nurse, P. (1986). cdc25+ functions as an inducer in the mitotic control of fission yeast. Cell *45*, 145–153.
38. Hiraoka, Y., Toda, T., and Yanagida, M. (1984). The NDA3 gene of fission yeast encodes beta-tubulin: a cold-sensitive nda3 mutation reversibly blocks spindle formation and chromosome movement in mitosis. Cell *39*, 349–358.
39. Rosenberg, J.A., Tomlin, G.C., McDonald, W.H., Snydsman, B.E., Muller, E.G., Yates, J.R., 3rd, and Gould, K.L. (2006). Ppc89 links multiple proteins, including the septation initiation network, to the core of the fission yeast spindle-pole body. Mol. Biol. Cell *17*, 3793–3805.
40. Alfa, C.E., Booher, R., Beach, D., and Hyams, J.S. (1989). Fission yeast cyclin: subcellular localisation and cell cycle regulation. J. Cell Sci. Suppl. *12*, 9–19.
41. Booher, R., and Beach, D. (1988). Involvement of cdc13+ in mitotic control in Schizosaccharomyces pombe: possible interaction of the gene product with microtubules. EMBO J. *7*, 2321–2327.
42. Furge, K.A., Cheng, Q.C., Jwa, M., Shin, S., Song, K., and Albright, C.F. (1999). Regions of Byr4, a regulator of septation in fission yeast, that bind Spg1 or Cdc16 and form a two-component GTPase-activating protein with Cdc16. J. Biol. Chem. *274*, 11339–11343.

43. Beltraminelli, N., Murone, M., and Simanis, V. (1999). The S. pombe zfs1 gene is required to prevent septation if mitotic progression is inhibited. J. Cell Sci. *112*, 3103–3114.
44. Murone, M., and Simanis, V. (1996). The fission yeast dma1 gene is a component of the spindle assembly checkpoint, required to prevent septum formation and premature exit from mitosis if spindle function is compromised. EMBO J. *15*, 6605–6616.
45. Kinoshita, K., Nemoto, T., Nabeshima, K., Kondoh, H., Niwa, H., and Yanagida, M. (1996). The regulatory subunits of fission yeast protein phosphatase 2A (PP2A) affect cell morphogenesis, cell wall synthesis and cytokinesis. Genes Cells *1*, 29–45.
46. Kemp, H.A., and Sprague, G.F., Jr. (2003). Far3 and five interacting proteins prevent premature recovery from pheromone arrest in the budding yeast Saccharomyces cerevisiae. Mol. Cell. Biol. *23*, 1750–1763.
47. Castets, F., Bartoli, M., Barnier, J.V., Baillat, G., Salin, P., Moqrich, A., Bourgeois, J.P., Denizot, F., Rougon, G., Calothy, G., and Monneron, A. (1996). A novel calmodulin-binding protein, belonging to the WD-repeat family, is localized in dendrites of a subset of CNS neurons. J. Cell Biol. *134*, 1051–1062.
48. Moreno, C.S., Park, S., Nelson, K., Ashby, D., Hubalek, F., Lane, W.S., and Pallas, D.C. (2000). WD40 repeat proteins striatin and S/G(2) nuclear autoantigen are members of a novel family of calmodulin-binding proteins that associate with protein phosphatase 2A. J. Biol. Chem. *275*, 5257–5263.
49. Schmidt, S., Hofmann, K., and Simanis, V. (1997). Sce3, a suppressor of the Schizosaccharomyces pombe septation mutant cdc11, encodes a putative RNA-binding protein. Nucleic Acids Res. *25*, 3433–3439.
50. Krapp, A., Cano, E., and Simanis, V. (2003). Mitotic hyperphosphorylation of the fission yeast SIN scaffold protein cdc11p is regulated by the protein kinase cdc7p. Curr. Biol. *13*, 168–172.
51. Jiang, W., and Hallberg, R.L. (2001). Correct regulation of the septation initiation network in Schizosaccharomyces pombe requires the activities of par1 and par2. Genetics *158*, 1413–1429.
52. Lahoz, A., Alcaide-Gavilán, M., Daga, R.R., and Jimenez, J. (2010). Antagonistic roles of PP2A-Pab1 and Etd1 in the control of cytokinesis in fission yeast. Genetics *186*, 1261–1270.
53. Le Goff, X., Buvelot, S., Salimova, E., Guerry, F., Schmidt, S., Cueille, N., Cano, E., and Simanis, V. (2001). The protein phosphatase 2A B′-regulatory subunit par1p is implicated in regulation of the S. pombe septation initiation network. FEBS Lett. *508*, 136–142.
54. Seshan, A., and Amon, A. (2004). Linked for life: temporal and spatial coordination of late mitotic events. Curr. Opin. Cell Biol. *16*, 41–48.
55. Goudreault, M., D'Ambrosio, L.M., Kean, M.J., Mullin, M.J., Larsen, B.G., Sanchez, A., Chaudhry, S., Chen, G.I., Sicheri, F., Nesvizhskii, A.I., et al. (2009). A PP2A phosphatase high density interaction network identifies a novel striatin-interacting phosphatase and kinase complex linked to the cerebral cavernous malformation 3 (CCM3) protein. Mol. Cell. Proteomics *8*, 157–171.